Introduction: Real-time monitoring of acquired resistance to targeted therapies may be possible via the detection of tumour mutations arising in circulating free deoxyribonucleic acid (DNA) in plasma. Droplet digital polymerase chain reaction (ddPCR) partitions a plasma sample into thousands of discrete amplification events, thereby allowing the detection of rare mutations. A mutation in the cetuximab-binding epitope of the epidermal growth factor receptor (EGFR; S492R) was recently identified that rendered cells resistant to cetuximab but not to panitumumab (Montagut et al, Nature Medicine 2012). The frequency of EGFR S492R mutations have not yet been evaluated in a large, controlled clinical trial. Here we characterised the in vitro activity of panitumumab and cetuximab on EGFR S492R and examined the frequency of this mutation in patients included in the ASPECTCT study.

Methods: The binding of panitumumab and cetuximab to wild-type (WT) EGFR or EGFR S492R was analysed in transduced CHO cells by flow cytometry (FC). The activity of these agents on WT EGFR and EGFR S492R was also assessed by immunoblotting (IB). ASPECTCT is a randomised, non-inferiority phase III monotherapy trial of panitumumab vs cetuximab in the treatment of patients with metastatic colorectal cancer (mCRC). Patient plasma samples from ASPECTCT were collected at pre-treatment, week 7 and 30 days post-treatment. ddPCR was conducted using the Bio-Rad QX100 system, with multiplexed custom primer/probes to C1476A, C1476G and A1474C and to WT EGFR. This assay can detect the presence of EGFR S492R in plasma with 99% probability using a limit of detection of 0.2% at high DNA input and 1% at low DNA input. The false positive rate was approximately 0.006%.

Results: Both FC and IB data showed that panitumumab, but not cetuximab, bound to and inhibited the activation of EGFR S492R. Overall, 999 patients were randomised and treated in ASPECTCT and post-treatment samples were available for EGFR codon 492 assessment from 53% of patients (261/496) treated with panitumumab and 57% of those (285/503) treated with cetuximab. The EGFR S492R mutation was detected in 1% (95% confidence interval [CI]: 0%-3%) of patients (3/261) treated with panitumumab compared with 16% (95% CI: 12%-21%) of those (46/285) treated with cetuximab (p < 0.001). Mutational analysis of pre-treatment samples from patients included in the ASPECTCT study is ongoing.

Conclusion: Using a sensitive analysis technique (ddPCR), EGFR S492R was detected in 1% of mCRC patients treated with panitumumab and 16% of patients treated with cetuximab in a large, randomised controlled phase III trial. Further research is needed to determine the clinical implications of these findings.

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